

Clonal propagation of *Bambusa vulgaris* by leafy branch cuttings

M. S. Islam • M. K. Bhuiyan • M. M. Hossain • M. A. Hossain

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Abstract: *Bambusa vulgaris* Schrad ex wendl is a widely cultivated bamboo species in rural Bangladesh for its versatile uses. The vegetative propagation becomes the only viable alternative for this species because *B. vulgaris* does not set seed after sparse flowering, which makes seedling progenies unavailable. A low-cost propagation trial was conducted to explore the clonal propagation techniques for the species with two types of small branch cuttings, nodal leafy cuttings and tip cuttings. The cuttings, after treating with 0, 0.1%, 0.4%, and 0.8% IBA solutions, were kept in non-mist propagator to let them to root for assessing the rooting ability. The cuttings were rooted in four weeks and were allowed to grow in the polybags for 10 months under nursery condition to assess their steckling capacity. The study reveals that both types of branch cuttings are able to develop roots, shoots, to survive and to form rhizome under the nursery condition. Rooting ability of the cuttings was significantly enhanced by the application of rooting hormone - IBA. The highest rooting percentage in nodal leafy cuttings and the tip cuttings (56.67% and 51.0%, respectively) were observed in 0.8% IBA treatment, followed by 0.4% IBA and the lowest (34.3% and 30.0%, respectively) was in control. The highest number of root developed per cutting (9.77 and 8.33 in nodal leafy cuttings and the tip cuttings, respectively) was also obtained from the cuttings treated with 0.8% IBA solution, followed 0.4% IBA treatment and the lowest (3.1 and 2.1, respectively) was in the cuttings without treatment. However, the length of the longest root varied significantly neither with the cutting types nor the concentrations of IBA solution. Survival percentage of the stecklings in nursery condition was significantly enhanced by IBA.

Keywords: *Bambusa vulgaris*; leafy branch cuttings; non-mist propagator; rooting ability; steckling capacity

Introduction

Bamboos of Poraceae family and Bambosidae sub-family are the fastest growing woody species. These multipurpose plants (Shanmughavel et al. 1997) play vital roles in every-day life of millions of people living in the South-East Asia by meeting their basic needs in the forms of food, fodder, fuel, clothing, medicine, shelter and raw materials for industries including paper and pulp, furniture, construction etc. Their versatile use (Banik et al. 1997) is due to their excellent splitting ability (Banik 2002), tensile and compressive strength, amenability of being harvested within five years after planting (Negi 1996), etc. With the swelling human population, the demands for bamboos in housing, agricultural activities and paper industries are increasing. However, the area and quantity of bamboo stocks in Bangladesh are declining alarmingly due to the destruction of tropical forests, indiscriminate harvesting associated with increasing demand, lack of proper knowledge and suitable technique for cultivation (Banik 1995). It is, therefore, an urgent need to develop and maintain the bamboo resource bases through massive plantation program with genetically improved planting materials.

Among 75 genera and 1250 species in the world (Sharma 1980), 33 species are grown in Bangladesh - of which seven occur naturally in forests and the rest are cultivated in homestead throughout the country (Banik 1980). *Bambusa vulgaris* Schrad ex wendl is the most prevalent and preferred bamboo species in rural homesteads in Bangladesh. However, flowering is sparse in the species (Koshy and Pushpangadan 1997) and the flowering that is seen is not good enough to set viable seeds due to the cumulative effect of a number of physical and physiological factors (Banik 1979; Koshy and Jee 2001). This results in the inadequacy of seedling progenies for the species (McClure 1966). Consequently, the knowledge on alternative means of obtaining propagation materials became inevitable wherein vegetative propagation methods for the species fits perfectly (Banik 2000; Koshy and Pushpangadan 1997).

Rhizome cutting, offset planting, culm or stem cutting, branch cutting are the various approaches of vegetative propagation for bamboos with known issues for some of them like the low rate of multiplication, need for skilled workforce, or high capital in-

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M.S. Islam • M.K. Bhuiyan • M. M. Hossain • M.A. Hossain (✉)
Institute of Forestry and Environmental Sciences, University of Chittagong, Chittagong-4331, Bangladesh.

Email: aktar_forestry@hotmail.com

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vestments etc. Among these approaches, rhizome cutting is popular in the villages but the lower rate of survival and bulkiness (4–30 Kg) of rhizomes makes it an expensive option in terms of obtaining and transporting propagules and the limited availability of rhizomes due to the risk of detrimental effect of removing rhizomes on the regeneration potential of the source clump. Pre-rooted and pre-rhizomed branch cuttings (Banik 1989) are the other good choices, however, pre-rooted branch cuttings is limited only to the wet seasons and very few branches attached with the mother culms can develop root at their base which makes it inapplicable for large-scale propagule production. The recently developed branch-cutting technique can overcome many of these problems (Seethalakshmi et al. 1983) as it is inexpensive, produces a bulk of propagation materials with high survival potential in short time and reduces the labor and transportation cost (Banik 2000). A branch with the base or one node from the main branch and 3–4 nodes from secondary branches is cut for propagation (Banik 2000). Our previous studies (Hossain et al. 2005; Hossain et al. 2006) have shown that the cuttings start to develop active buds within 7–10 days and produce profuse roots in the propagation beds within 4–8 weeks, depending on the season. However, the number of suitable branches (larger than 5 mm diameter with base or node) available from a clump to produce root in the propagation bed is inadequate and the branch cutting method limited only to rainy season during June–July. Our approach can overcome the issue by using small, leafy branch cutting for vegetative propagation to produce cuttings in high quantity from a single clump, inexpensively round the year. In the present study we studied the rooting ability of small leafy branch cuttings (around 20 cm in length and 3 mm in diameter) in non-mist propagator with or without rooting hormone IBA and their steckling capacity in the nursery condition, with a objective to obtain large number of propagules from a single clump of bamboo.

Materials and methods

Study area and climatic conditions

The study was conducted from June 2005 to November 2006 in the nursery of Institute of Forestry and Environmental Sciences, University of Chittagong, Bangladesh. The site lies at 22°27'27" N and 91°48'30" E and enjoys typical tropical monsoon with hot humid summer and cool dry winter. The mean monthly temperature is between 21.8°C and 29.2°C and the maximum and minimum is 26°C and 15°C, respectively. Relative humidity is lowest (64%) in February and highest (95%) in June to September; mean annual rainfall is about 300 cm, which occurs mostly between June and September (Gafur et al. 1979). Mean monthly day length varies between 10 h 35 min in December and 13 h 20 min in June (Hossain et al. 2005).

Clump selection and preparation of cuttings

Vigorous five-year old clumps were selected based on 1) matur-

ity in terms of their ability to produce sufficient number of branches, 2) growth potential in terms of the number of culms per clump, height, diameter and length of internodes, and 3) disease and pest-freeness. Cuttings were collected from the selected branches of the pre-selected clumps by excising small secondary or tertiary branches. Two types of small branch cuttings were made - nodal leafy cuttings and tip cuttings. The nodal leafy cuttings were the small branches with one node from the primary or secondary branch (approximately 2 cm from both sides of node as the base of the leafy branch) along with 3–4 nodes and fleshy leaves with tip (Fig. 1). Tip cuttings were the healthy leading shoot apex along with 2–3 nodes of the secondary or tertiary branch without swollen base. Average lengths of nodal leafy cuttings and tip cuttings were 25.7 cm to 28.3 cm and 18.94 to 20.97 cm, respectively, while the diameter was 4.6 mm to 5.4 mm and 2.24 to 3.05 mm in nodal leafy cuttings and tip cuttings, respectively (Fig. 1). The lengths and diameters of the cuttings under various treatments within the cutting type were kept nearly uniform to avoid on-treatment variations.



Fig. 1 Nodal leafy cuttings with base (left) and tip cuttings (right) of *B. vulgaris* are ready for rooting trial.

A total of 480 cuttings, taking 240 of each type, were used for the rooting trial in the study. Cuttings were immersed briefly into a solution of fungicide, Diathane M45 (Rohm & Co. Ltd., France; 2 g·L⁻¹) to avoid fungal infection. Then they were rinsed and kept under shade for 10 min in open air. Finally the cuttings of each type were treated with 0, 0.1%, 0.4%, and 0.8% (w/v) Indole 3-Butyric Acid (IBA) solution to assess the effect of applied rooting hormone on rooting ability of cuttings by dipping the base of the cuttings briefly into the solution.

Rooting trials

The cuttings were planted in the perforated plastic trays filled with coarse sand mixed with gravel. Each tray contained 10 cuttings which made 60 replicate cuttings for each of the treatment (six trays; 10 cuttings in each tray). Trays containing the cuttings were placed in the non-mist propagator (Kamaluddin 1996) for rooting (Fig. 2). About 85%–90% humidity was maintained within the propagator. Every day the propagator was opened briefly in the early morning and the late afternoon for gaseous exchange to avoid excessive heat accumulation. Again, the propagator was kept under bamboo made shed to avoid excessive heat on the propagator. Further shading was achieved by putting jute mat over the roof of the shed. Thus the photosynthetic photon flux inside the propagator was reduced to about 12% of full

sun. During the experiment, mean maximum temperature was 32°C and the mean minimum temperature 25°C.

Four weeks after setting the experiment, the rooted cuttings were subjected to weaning before transferring to polybags, particularly towards the end of the rooting period during root lignifications. For weaning the shed was kept open at night for three days and then day and night for another three days.

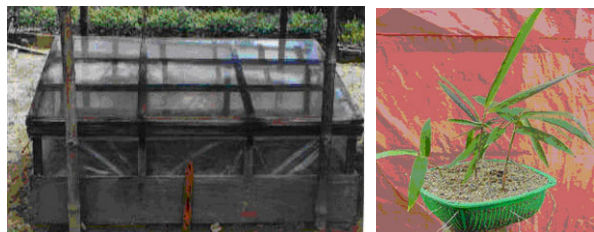


Fig. 2 Non-mist propagator (left) and the cuttings rooted in perforated plastic trays filled with coarse sand mixed with gravel in the propagator (right).

Transferring of rooted cuttings

The rooted and weaned cuttings were then transferred to the polybag (25 cm × 15 cm) filled with soil and decomposed cow dung at a ratio of 3:1 and were placed in the nursery bed. They were allowed to grow for 10 months for assessing the steckling capacity of the cuttings developed under various treatments. Proper care and maintenance were ensured from the time of setting the experiment up to the final assessment. During transferring the rooted cuttings from the rooting medium to the growth medium (polybags filled with soil and decomposed cowdung) the rooting percentage, root number, root length, cutting length and cutting diameter of each cutting were recorded. Survival percentage of the cuttings was assessed by counting the number of rooted cuttings survived and thrived 10 months after transferring them into the poly bags under the nursery condition.

Data analysis

All data were analyzed with Microsoft Excel and SPSS ver.13.0 (SPSS Incorporation, Chicago, USA). Possible treatment variations were explored by analysis of variance (ANOVA) and Duncan multiple range test (DMRT). Rooting percentage values were adjusted accordingly by using arc sign root square before putting the data into analysis since the percentage of cuttings rooted were distributed between the range of 30 to 60 and proportions were based on equal denominator.

$$Y = \sin^{-1} (x)^{1/2}$$

where Y = Arc sign transformed value; x = Proportion of number of cuttings rooted to the number of cuttings substituted ($100 - 1/4 n$) where 'n' is the number of units upon which the percentage data is based *i.e.*, the denominator used in compiling the percent-

ages.

Results and discussion

Rooting ability of cuttings

Rooting percentage

Rooting percentage of *B. vulgaris* branch cuttings varied from 36.7 to 56.7 in nodal leafy cuttings and 30.0 to 51.7 in tip cuttings. Exogenous rooting hormone IBA and its various concentrations affected the rooting percentages of cutting types (nodal leafy cuttings and tip cuttings) significantly. In the nodal leafy cuttings, the highest rooting percentage (56.7%) was observed in the cuttings treated with 0.8% IBA solution followed by the cuttings treated with 0.4% IBA (48.3%) and the lowest (36.7%) was in control (Fig. 3). The maximum rooting percentage (56.7%) in tip cuttings was also obtained from the cuttings treated with 0.8% IBA solution followed by 0.4% IBA (42.3%) and the minimum rooting percentage (30.0%) was in cuttings without any treatment (Fig. 3). However, the rooting percentage in nodal leafy cuttings was significantly higher than the tip cuttings among the treatments.

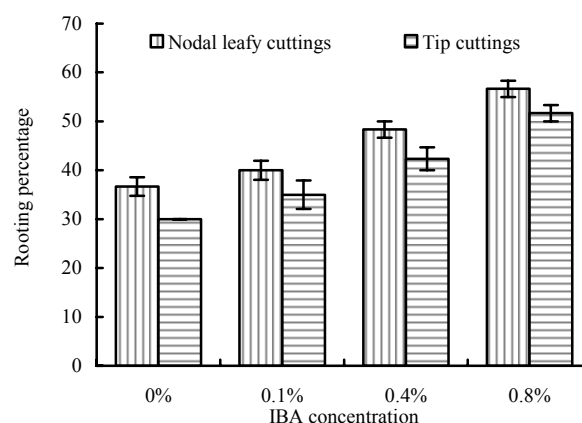


Fig. 3 Rooting percentage of nodal leafy cuttings and tip cuttings of *B. vulgaris* under various treatments. Same letters indicate no significant difference at $p < 0.05$ (ANOVA and DMRT). Bar indicates the standard error of means.

In the present study, rooting percentage of branch cuttings of *B. vulgaris* varied significantly among the treatments with the varying concentrations of IBA solution. This finding is validated by several reports the influence of IBA on rooting percentage of bamboo branch cuttings. For instance, Somashekar et al. (2004) reported the highest rooting percentage (85%, in leafy branch cuttings with tip and 80% in nodal cuttings) in the cuttings treated with 2,500 ppm IBA. Hossain et al. (2005) observed that rooting ability of cuttings and growth performance of cuttings were affected significantly by IBA treatment. They recorded the highest rooting percentage (84%) in 0.2% IBA treated cuttings compared to untreated cuttings (73.3%). In a separate experiment, Hossain et al. (2006) reported that the highest percentage of root-

ing (63.33%) was observed in the *B. vulgaris* var. *striata* branch cuttings treated with 0.4% IBA followed by 0.2% IBA (60%) and the lowest (30%) was in the cuttings without treatment. Sharma (1980) reported that hormone treatment accelerated the success of rooting in branch cuttings and the rooting percentage was 80% in *B. vulgaris*. Moreover, Surendran and Seethalakashi (1985) reported significant enhancement in rooting and sprouting responses of bamboos by the application of growth regulators - IBA and NAA. Sing et al. (2002) found that application of IAA, IBA, NAA, NOA either alone or in combinations have influence on rooting percentage from culm and culm branch cutting as they augment the endogenous level of auxins promoting early and high rate of root induction as compared to the control. In cuttings with low endogenous auxin contents, applied auxin causes significant increases in both rooting percentages and number of roots (Bowen et al. 1975).

Number of roots per cutting

Number of roots per cutting varied from 3.1 to 9.8 in nodal leafy cuttings and 2.1 to 8.3 in tip cuttings. In nodal leafy cutting, the highest number of roots (9.8) was observed in the cuttings treated with 0.8% IBA, followed by the cuttings treated with 0.4% IBA (7.0) and the lowest number of roots (3.1) was in cuttings without treatment (Figs. 4 and 5). Similarly, in tip cuttings, the highest number of roots (8.3) was obtained in cuttings treated with 0.8% IBA solution followed by 0.1% IBA (7.3) and the lowest (2.1) was in untreated cuttings (Figs. 4 and 5). The number of root developed in the nodal cuttings was significantly higher than the tip cuttings in all concentrations of IBA treatment.

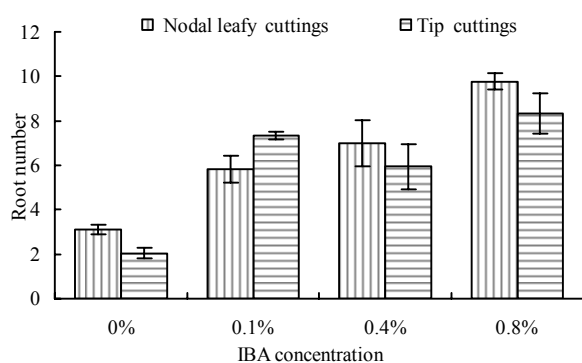


Fig. 4 Number of root developed in nodal leafy cuttings and tip cuttings of *B. vulgaris* treated with various concentration of IBA solution. Same letters indicate no significant difference at $p < 0.05$ (ANOVA and DMRT). Bar indicates the standard error of means.

In both types of cuttings, the number of roots were significantly increased by IBA treatments; this result strengthens previous reports, for instance, Hossain et al. (2005) observed significant increase in the number of roots in IBA treated cuttings (6.8 to 7.9 in 0.2% IBA treated cuttings) compared to in untreated cuttings (4.1 to 6.6). In a separate work, Hossain et al. (2006) reported that the number of roots in the branch cutting of *B. vulgaris* var. *striata* was the maximum for 0.4% IBA treatment followed by 0.2% IBA treatment and the lowest was in the con-

trol cuttings. Similar result was reported by Castillo (1990) as they observed the maximum number of roots in base cuttings treated with 1,000 mg·L⁻¹ IBA. Kamaluddin et al. (1996) reported a significant increase in root number as well as rooting percentage with the application of IBA in vascular plant, *Artocarpus heterophyllus* and recorded the highest number of roots (9.4) in cuttings treated with IBA.



Fig. 5 Rooting ability of nodal leafy cuttings (left) and tip cuttings (right) of *B. vulgaris* treated with different concentration of rooting hormone IBA.

Applied auxin is known to intensify root-forming process in cuttings. For instance, polysaccharide hydrolysis is activated under the influence of applied IBA, and as a result, the contents of physiologically active sugar increases providing materials and energy for meristematic tissues and later for root primordia and roots in cuttings of vascular plants (Ermakove and Zhuravieva 1976). Hassig (1983) examined the function of endogenous root forming components of vascular plants and demonstrated that auxin is required for the development of callus in which root primordia are initiated.

Root length of cuttings

Average lengths of the longest roots in nodal leaf cuttings were within the range between 10.1 cm and 14.0 cm and in tip cuttings between 9.9 cm and 12.1 cm without any significant difference in the average lengths of the longest roots among the treatments or between the cutting types. However, in nodal leafy cuttings, the highest root lengths (14.0 cm) was obtained from the cuttings treated with 0.4% IBA solution followed by the 0.1% IBA (13.9 cm) and the lowest (10.1 cm) was found in untreated cuttings (Table 1 and Fig. 6). Interestingly, the highest root lengths in tip cuttings (12.1 cm) was observed in the cuttings treated with 0.1% IBA solution followed by 0.4% IBA (12.0 cm) and the lowest (8.2 cm) was found in cuttings treated with 0.8% IBA solution (Table 1 and Fig. 5) which was totally reverse compared to nodal leafy cuttings. Further experiment is needed to explain the reason behind this total contrast between nodal leafy cutting and tip cuttings. Hossain et al. (2006) reported earlier that in *B. vulgaris* var. *striata* branch cuttings, average length of the longest roots was maximum for 0.2% IBA treatment followed by 0.4% IBA treatment and the lowest was in the control.

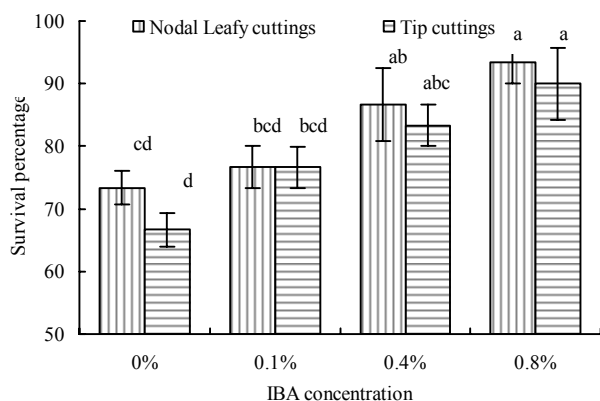


Fig. 6 Survival percentage of nodal leafy cuttings and tip cuttings of *B. vulgaris* ten months after transforming the rooted cuttings into the polybags. Same letters indicate no significant difference at $p < 0.05$ (ANOVA and DMRT). Bar indicates the standard error of means.

Table 1. Average length (cm) of roots developed in the cuttings under various concentration of IBA solution five weeks after setting the cuttings in the propagators

Cutting types	IBA concentration				p
	0	0.1%	0.4%	0.8%	
Nodal leafy cuttings	10.1±0.41 ^a	13.9±0.95 ^a	14.0±0.74 ^a	12.8±2.03 ^a	NS
Tip cutting	10.2±0.70 ^a	12.1±2.09 ^a	12.0±1.68 ^a	9.9±0.44 ^a	NS

Same superscript letters indicate no significant difference at $p < 0.05$ (ANOVA and DMRT). ± indicates the standard error of means while NS for not significant at $P < 0.05$ (ANOVA and DMRT).

Steckling capacity

The survival percentage of rooted cuttings of *B. vulgaris* in the nursery was observed significantly higher in the cuttings treated with IBA than control. In nodal leafy cuttings and tip cuttings, survival percentages were 73.3 to 93.3 and 66.7 to 90, respectively, three months after transferring the rooted cuttings in the polybags (Figs. 6 and 7). The highest survival percentage in nodal leafy cutting (93.3%) was observed in the cuttings with 0.8% IBA treatment followed by 0.4% IBA (86.7%) treated cuttings and the lowest (73.3%) was in untreated cuttings. In tip cuttings, the highest survival percentage (90%) was for 0.8% IBA treatment followed by 0.4% IBA treatment (83.3%) and the lowest (66%) was in control (Fig. 6). The cuttings were shown to grow vigorously with formation rhizome and new culms at the base 10 months after transferring them to polybags (Fig. 7 and Fig. 8).

The survival percentage of cuttings (rooted cuttings) was higher in IBA treated ones in both types of branch cuttings. The result of the present study was in line with the report by Hossain et al. (2005) who observed the highest survival percentage (95.2%) was in the cuttings rooted with IBA solution and the lowest (90%) in the controlled cuttings. Pattanaik et al. (2004) reported 100% field survivals of *B. balcooa* cutting treated with

200 mg·L⁻¹ IBA two years after field planting. However, Hossain et al. (2006) reported that the survival percentage did not vary between the cutting types and the concentrations of rooting hormones in the rooted branch cuttings of *B. vulgaris* var. *striata*. The higher survival potential in the cuttings rooted with IBA solution over the control cuttings was due to the higher number of roots produced in the cuttings treated with IBA as evident in Figs. 5 and 6. However, no report was found to discuss the inner mechanism behind such augmentation in the survival percentage of the rooted cuttings of bamboo, which can be an interesting field of further investigation.



Fig. 7 Steckling performance of leafy cuttings (left) and the tip cuttings (right) of *B. vulgaris* three months after transferring the rooted cuttings in the polybags.



Fig. 8 Cuttings of ten months old developed from nodal leafy branch cuttings (left) and tip cuttings (right) are ready for out planting.

Conclusion

Rooting ability of two types of branch cuttings (nodal leafy cuttings and tip cuttings) of *B. vulgaris* was investigated under four concentrations of IBA solution with respect to a control. The highest rooting percentage and the number of roots developed per cutting were observed in the cuttings treated with 0.8% IBA solution in both the types of cuttings and the lowest was in the cuttings without treatment. In steckling performance IBA treated cuttings showed better survival capacity in the nursery condition than control. The result of the present study could help in opening a new avenue for the propagation of this variety of multipurpose bamboo since both types of branch cuttings showed potential in developing roots, in survival and in rhizome formation. However the rooting percentage of leafy branch cuttings of *B. vulgaris* in this study was 56% and we believe that there is fur-

ther scope of increasing the rooting ability for the species. Furthermore, the performance of planting stock developed through these methods was not assessed in the field due to the limitations in time for the study. This might be one of the important aspects for future study.

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